to actin, whereas fast myosins such as muscle myosin-2 and some class-1 myosin members have a low duty ratio (< 0.1). The members of the respective groups are assumed to use different ways to couple conformational changes at the nucleotide binding regions to changes that occur at the actin binding sites. Conserved active-site elements termed switch-1 and switch-2 play a major role in the coupling mechanism; however, whether and to which extent small variations in the sequence of switch-1 and switch-2 affect the duty ratio of a given myosin, thus determining its ability for processive movement, fast contractility or tension bearing remains unresolved. Based on structural considerations and confirmed by mutational analyses, we identified key residues in the nucleotide binding pocket that are responsible for making ADP dissociation kinetics dependent on the concentration of free magnesium ions. The exchange of a single amino acid in switch-2 affected the motor properties of all myosins tested, but also transformed low duty ratio motors into high duty ratio and vice versa. In addition, x-ray structural analyses and molecular modeling allowed us to relate the observed changes to altered coupling between the active sites and actin binding regions. These results, together with our cell biological studies demonstrating for the first time that magnesium ions have a regulatory role on motor protein function in vivo reveal that, switch-2 can act as magnesium sensor critically determining the mechanochemical properties of myosins.

22-Subg
From Single Molecule Fluctuation to Muscle Contraction: A Brownian Model of A.F. Huxley's Hypotheses
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Adaptive force generation of muscle in response to external stimuli is the result of thermally fluctuating, cyclical interactions between myosin and actin, which together form the actomyosin complex. Normally, these fluctuations are modelled using transition rate functions that are based on muscle fiber behaviour, in a phenomenological fashion (Huxley , 1957; Huxley & Simmons, 1971). However, such a basis reduces the predictive power of these models. As an alternative, we propose a model which uses direct single molecule observations of actomyosin fluctuations (Kitamura, et al. Nature 1999, BIOPHYSICS, 2005; Iwaki et al. Nat. Chem Biol. 2009). We precisely estimate the actomyosin potential bias and use diffusion theory to obtain a Brownian ratchet model that reproduces the complete cross-bridge cycle. The model is validated by simulating several macroscopic experimental conditions, while its interpretation is compatible with two different force-generating scenarios (Lorenzo & Yanagida, PloS One, 2012).

23-Subg
Watching Individual DNA Helicases and Motor Proteins Behaving and Misbehaving
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We can now watch individual proteins acting on single molecules of DNA. Using these approaches, we have been imaging the translocation of motor proteins, such as the ssDNA translocases and DNA helicases, RecBCD, RecQ, and Sgs1, and the dsDNA translocases and chromatin-remodeling enzymes, Rad54 and Tid1 proteins.
The RecBCD enzyme comprises two motor subunits: RecB, a 3'→5' SF1-helicase, and RecD, a 5'→3' SF1-helicase. Although the mean unwinding rate of individual RecBCD enzyme molecules is close to ensemble measurements, the rates of individual enzyme molecules vary widely. Individual RecBCD molecules unwind DNA at constant rates, suggesting static, not dynamic, disorder. This apparent conformational heterogeneity is static on the experimental time scale of DNA unwinding. We discovered that transiently halting a single enzyme-DNA complex changes the rates of the RecBCD molecule. We will demonstrate that the behavior of individual RBCD enzymes manifests a basic tenet of the ergodic hypothesis, suggesting that ligand binding kinetically traps a single conformer that is capable of redistributing to all other states within the population of molecules at equilibrium.
Using TIRF microscopy, the helicase activity of RecQ was visualized on single molecules of DNA using a fluorescent ssDNA sensor. By monitoring the formation and progression of individual unwinding forks, we observe that both the frequency of initiation and rate of unwinding are highly dependent on RecQ concentration. We establish that unwinding forks can initiate internally by melting into dsDNA and can proceed in both directions. The findings suggest that initiation requires a RecQ dimer, and continued unwinding involves the repeated cooperative action of multiple monomers at the DNA unwinding fork. We propose a distinctive model wherein RecQ melts dsDNA internally to initiate unwinding, and subsequently unwinds DNA as a dynamic assembly of proteins cooperating at the fork.

24-Subg
Kinesin-14: A League of their Own
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Kinesin-14 represents a subfamily of kinesins that are nonprocessive, promote microtubule (MT) minus-end-directed force generation, and contain C-terminal motor domains that are dimerized through an N-terminal coiled-coil. Unlike the well-known N-terminal motor domain kinesins that use an asymmetric hand-over-hand mechanism for MT plus-end-directed processive stepping, Kinesin-14s use a MT minus-end-directed powerstroke to generate force to crosslink and slide one MT relative to another. While most Kinesin-14s are homodimers like Drosophila Ncd, S. cerevisiae Kar3Vik1 is a heterodimer. The C-terminal domain of Vik1 exhibits the structural fold of a kinesin motor domain, binds MTs independent of Kar3, yet lacks a nucleotide-binding site. Furthermore, Kar3Vik1 binds across adjacent MT protofilaments, a non-canonical MT binding configuration. The results indicate that Kar3Vik1 collides with the MT through Vik1, promoting MT binding by Kar3. The tight binding of Kar3 destabilizes the Vik1 interaction with the MT, positioning Kar3Vik1 for the start of the powerstroke. Rapid ATP binding to Kar3 is associated with the rotation of the coiled-coil stalk, and post-powerstroke ATP hydrolysis is independent of Vik1 providing additional evidence that Vik1 rotates with the coiled-coil during the powerstroke. Detachment of Kar3Vik1 from the MT completes the cycle and allows the motor to return to its initial conformation. Supported by NIH GM54141.

Subgroup: Nanoscale Biophysics

25-Subg
Single Molecule Enzymology
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We use single molecule force-clamp techniques to study the activity and chemical mechanisms of oxidases and oxidoreductase enzymes. In this approach the length of an extending protein is measured while the pulling force is actively kept constant with a feedback loop. Using the force-clamp technique we have investigated the force-dependency of protein folding, unfolding and of chemical reactions. For example, using various types of force pulses we can drive a single substrate protein to well defined extended states and monitor the reduction of its disulfide bonds by thioredoxin enzymes, with sub-Angstrom resolution (1). By varying the pulling force on the substrate we can also identify different chemical mechanisms of reduction. Using maximum likelihood techniques we have resuscitated ancient thioredoxin enzymes, going back billions of years. We have studied the activity of our resuscitated enzymes using our single molecule techniques in an effort to observe the evolution of the chemical mechanisms of reduction over time(2). Finally, we have used these single molecule techniques to study the activity of oxidases such as PDI and DsbA, and of glutharedoxin(3). Our results demonstrate that single molecule force-spectroscopy techniques provide an entirely novel and powerful approach to study enzymes, at an unprecedented resolution. These experiments show that force-clamp AFM probes dynamic rearrangements within an enzyme's active site, which cannot be resolved by any other current structural biological technique. We anticipate that these studies will be extended to a wide range of other enzymes.
1.Wiita et al, (2007) Probing the chemistry of thioredoxin catalysis with force. *Nature*, 450:124-7.
2.Perez-Jimenez, et al, (2011). Single-molecule paleoenzymology probes the chemistry of resurrected enzymes. *Nat Struct Mol Biol,* 18(5): 592-596.
3.Kosuri, P et al, (2012). Protein folding drives disulfide formation. *Cell,* in press

26-Subg
Dynamics of the Ribosomal Subunit Interface during TRNA Translocation at Near-Atomic Resolution
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During protein synthesis, movement of the transfer RNAs during translocation involves large structural motions of both the ribosome and the tRNAs. In particular, large scale collective inter-subunit rotations occur. Despite this rotation, the two ribosomal subunits remain bound during translocation via contact patches on the surface of the subunits. Combining crystal and cryo-EM structures with all-atom explicit solvent molecular dynamics simulations, we have characterised intermediate states and conformational motions of spontaneous tRNA translocation at near-atomic resolution. Systematic analysis of the dynamics of all inter-subunit contact patches suggest mechanisms by which the subunits communicate, and how the two subunits maintain their fine-tuned